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Comparison of two commercial ovine Campylobacter vaccines and an experimental bacterin in guinea pigs inoculated with Campylobacter jejuni

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Abstract

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Animals—51 female guinea pigs.

Procedures—Pregnant and nonpregnant animals were randomly assigned to 1 of 4 treatment groups and administered a commercial *Campylobacter* vaccine labeled for prevention of campylobacteriosis in sheep via two 5-mL doses 14 days apart (vaccine A; n = 13), another labeled for prevention of campylobacteriosis via two 2-mL doses (vaccine B; 12), an experimental bacterin prepared from the challenge strain (12), or a sham vaccine (14). Ten days later, animals were challenged IP with *C jejuni* IA3902; 48 hours later, animals were euthanized, complete necropsy was performed, and blood and tissue samples were obtained for bacteriologic culture.

Results—Administration of vaccine B or the experimental bacterin, but not vaccine A, significantly reduced 48-hour infection rates versus administration of the sham vaccine. A significantly reduced 48-hour infection rate was associated with administration of vaccine B independent of pregnancy status.

Conclusions and Clinical Relevance—Administration of vaccine B significantly reduced infection in guinea pigs challenged with *C jejuni* IA3902, similar to a homologous bacterin. Results suggested that vaccine B or an autogenous product may be effective in controlling ovine campylobacteriosis caused by this emergent abortifacient strain. Bacteriologic culture of blood, liver, bile, and uterus in nonpregnant guinea pigs 48 hours after inoculation may be a useful screening tool for comparing efficacy of *C jejuni* vaccines.

Disciplines

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Campylobacter spp are a well-recognized and notable cause of ovine abortion worldwide, with abortion rates of 5% to 50% reported in affected flocks.[1] A recent national survey ranked campylobacteriosis first among infectious causes of abortion in domestic sheep.[2] Results of 2 recent studies[3,4] indicate that *Campylobacter jejuni* is the predominant species responsible for *Campylobacter* spp–associated sheep abortion in the United States, and molecular typing techniques further reveal that within these *C jejuni*, a single tetracycline-resistant clone (named clone SA for sheep abortion) has emerged as the predominant cause of ovine abortion in Iowa, South Dakota, Idaho, California, Oregon, and Nevada.[4] This new emerging clone is important in that *Campylobacter* spp associated with ovine abortions in different regions and during different lambing seasons traditionally have marked genetic and antigenic heterogeneity.[3,5–9]

Abbreviation

OD Optical density

The effectiveness of vaccination for the prevention of ovine campylobacteriosis has been well documented[10–13]; however, experimental studies[12,14] have revealed inadequate protection to homologous challenge in ewes vaccinated with commercial vaccines of questionable efficacy and have also demonstrated inadequate cross protection in those vaccinated and later challenged with heterologous serotypes. Additionally, *Campylobacter* spp abortions have been reported in field cases from flocks that received commercial monovalent[15] and bivalent[3] vaccines. Accordingly, no single vaccine will likely be protective against all

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abortifacient *Campylobacter* spp, and continual efficacy testing is imperative to ensure the ongoing usefulness of a given vaccine. Use of pregnant guinea pigs is effective for screening abortifacient *Campylobacter* spp[16–18] and has been described for testing the efficacy of commercial *Campylobacter* vaccines intended for cattle[19,20] and sheep[20] as well as the efficacy of experimental bacterins.[21,22] Those efficacy studies reveal that vaccination with a monovalent bacterin of either *Campylobacter fetus* subsp *fetus* or *C jejuni* is not cross protective against challenge with the opposite species[22] and that protection is lower when challenge is with a heterologous strain of the same species.[21,22] These findings strongly support the need for polyvalent vaccines in the prevention of ovine campylobacteriosis.

In the United States, there are presently 2 commercially available, ovine-labeled polyvalent *Campylobacter* vaccines,[a,b] each containing *C fetus* subsp *fetus* and *C jejuni* strains. Given the emergence of *C jejuni* clone SA as the predominant *Campylobacter* sp associated with sheep abortions across multiple states,[4] we hypothesized that one or both of these vaccines may not be protective against this highly abortifacient strain. Previous studies[19–22] have assessed the prevention of abortion in pregnant guinea pigs following IP challenge as the evaluation endpoint for *Campylobacter* vaccine efficacy; however, given the increased cost associated with acquisition of pregnant animals and the housing costs associated with a multiweek observational study, we sought to determine whether the use of a mixed population of pregnant and nonpregnant females would be adequate and cost-effective for screening *Campylobacter* vaccines. Because bacteremia is a requisite step in the pathogenesis of nonvenereal *Campylobacter*-induced abortion,[23] the presence of blood and tissue infection in nonpregnant animals should provide an indication of the potential for abortion. In a previous study,[18] we observed abortions in pregnant guinea pigs as early as 48 hours following IP inoculation with *C jejuni* IA3902 (a clinical abortion isolate belonging to clone SA), and organisms were recoverable from the blood, bile, uterus, and fetoplacental units at that time. Accordingly, in the study reported here, blood and tissue colonization at 48 hours was selected as the evaluation endpoint to assess the efficacy of 2 commercially available ovine campylobacteriosis vaccines and an experimental homologous bacterin in preventing infection following IP challenge with *C jejuni* IA3902 in pregnant and nonpregnant guinea pigs. The purpose of the present study was to design and evaluate a procedure for screening *Campylobacter* vaccines and to use this procedure to assess the potential efficacy of the 2 commercially available ovine-labeled *Campylobacter* vaccines[a,b] against the emergent *C jejuni* clone SA.

Materials and Methods

Animals—All procedures were approved by the Institutional Animal Care and Use Committee of Iowa State University. Fifty-one female Hartley guinea pigs with a mean weight of 879 g were obtained from a commercial source.[c] All animals were recently bred, but pregnancy could not be guaranteed for animals purchased at this stage of gestation. Upon arrival, a rectal swab was obtained from each guinea pig, placed in transfer medium, and plated for *Campylobacter* culture as described. Guinea pigs were randomly assigned into 4 treatment groups (n = 14, 13, 12, and 12, respectively), housed in individual cages with wood chip bedding, and fed a commercial pelleted guinea pig diet ad libitum. Each guinea pig received the first dose of their respective vaccine treatment SC within 24 hours after arrival and received a second dose SC 14 days later. Ten days after receiving the second vaccination, guinea pigs were challenged IP with *C jejuni* IA3902.

Pregnancy determination—Abdominal palpation was performed to screen for pregnancy approximately 14 days after arrival. Pregnancy status was confirmed via abdominal ultrasonography 2 to 5 days prior to inoculation.

Vaccines—A commercial, polyvalent product[a] labeled for prevention of campylobacteriosis in sheep via two 5-mL doses administered SC (vaccine A) was used. For the purposes of the present study, the dose was reduced to 1 mL and administered SC in the interscapular region. Another commercial polyvalent product[b] labeled for prevention of campylobacteriosis in sheep via two 2-mL doses administered SC (vaccine B) was also used. To keep the effective dose the same as that used for vaccine A (20% of the labeled dose), the dose was reduced to 0.4 mL and administered SC in the interscapular region. The commercial products each contained *C fetus* subsp *fetus* and *C jejuni* strains in an aluminum hydroxide adjuvant. Accordingly, aluminum hydroxide gel[d] was used in preparing the homologous bacterin for this experiment. For preparation of the bacterin, a frozen stock of *C jejuni* IA3902 was grown microaerobically on Mueller-Hinton agar for 48 hours at 42°C, then subpassaged on Mueller-Hinton agar for an additional 24 hours at 42°C under the same atmospheric conditions. Colonies of *C jejuni* were harvested in PBS solution directly from plates by washing and then pelleted by centrifugation (15 minutes at 7,000 × *g*). The pellet was washed and resuspended in 10 mL of PBS solution containing 0.3% formalin. This suspension was centrifuged again (30 minutes at 7,000 × *g*), the supernatant discarded, and the wash process repeated 2 times. The final pellet was weighed and resuspended in sterile PBS solution to achieve a final concentration of 5 mg of *C jejuni* cells/mL. This final concentration was selected on the basis of a previous report[21] comparing antigen dose and various adjuvant types in which this concentration provided 93% protection against homologous challenge. A few drops of the diluted cells were streaked on Mueller-Hinton agar or added to enrichment broth, both of which were placed in a microaerophilic environment to evaluate efficacy of formalin killing. Once killing was verified, sterile $Al(OH)_3$ gel was added to the diluted cell suspension to achieve a final concentration of 0.2 mg of $Al(OH)_3$/mL. Higher concentrations of $Al(OH)_3$ adjuvant have been used in previous *Campylobacter* vaccine studies[14,22]; however, lower concentrations have been reported to be effective with experimental *Bordetella*[24] and *Salmonella*[25] vaccines, and a lower concentration was therefore selected to decrease the risk of adverse reactions. A hemocytometer was used to quantify the number of particles per milliliter for each preparation. The approximate concentrations for the 3 preparations were 6.29×10^7 particles/mL for vac-

cine A, 5.03 × 10^8 particles/mL for vaccine B, and 8.83 × 10^7 particles/mL for the experimental bacterin. The roughly 10-fold concentration of particles per milliliter in vaccine B, compared with the other vaccine and the bacterin, was further justification for its administration at a reduced dose, compared with the other treatments. The experimental bacterin was administered SC as a 1-mL dose in the interscapular region. A sham vaccine was prepared containing 0.2 mg/mL of $Al(OH)_3$ in sterile PBS solution and was administered SC as a 1-mL dose in the interscapular region.

Challenge-exposure inocula—Fresh bacterial cultures of *C jejuni* IA3902 (a clinical abortion isolate belonging to clone SA) were obtained following 24 hours of growth on Mueller-Hinton agar in anaerobic jars under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2) at 42°C. These cultures were collected in Mueller-Hinton broth, diluted to desired concentrations on the basis of optical density (ie, OD_{600}) to give approximately 1 × 10^6 CFUs/mL, and used as inocula in the challenge experiments. The final number of organisms in each suspension was determined via viable CFU counting. From the authors' laboratory experience, it was expected that 24-hour grown (midlogarithmic phase) *C jejuni* cultures would yield approximately 1 × 10^7 CFUs/mL when adjusted to an OD_{600} of 0.1.

Animal inoculation—Ten days after receiving their second vaccination, all animals were inoculated IP with 1 mL of 7.5 × 10^5 CFUs/mL to 1.0 × 10^6 CFUs/mL of Mueller-Hinton broth culture of *C jejuni* IA3902. This concentration was chosen on the basis of results from previous work[18] and a report[22] describing IP challenge with *C jejuni* in vaccinated pregnant guinea pigs.

Monitoring, euthanasia, and necropsy sampling—Once inoculated, guinea pigs were observed at least twice daily for signs of abortion or impending abortion. These signs included vaginal bleeding, presence of expelled fetuses, or visible fetal membranes. All animals were euthanized 48 hours after inoculation via IP injection of sodium pentobarbital (approx 150 mg/kg) and necropsied immediately. At necropsy, animals were inspected for gross lesions and samples were taken for bacterial culture and serologic and histologic examination. Samples harvested for *Campylobacter* isolation included heart blood, liver, bile, uterus, and all placental units. Samples of liver, gallbladder, uterus, and placenta were obtained for histologic examination and placed in 10% neutral-buffered formalin for 24 hours prior to paraffin embedding and routine processing for H&E staining.

Isolation and semiquantitative enumeration of *C jejuni* from necropsy samples—Heart blood and bile were collected and transported by use of sterile needles and syringes. Liver, uterus, and placenta were placed in separate sterile Petri plates for transport prior to bacteriologic culture. All samples were kept refrigerated until immediately prior to bacteriologic culture, which was performed on the day of collection. Fluid samples (blood and bile) were placed directly onto culture media and streaked by use of sterile cotton swabs. Tissue samples (liver, uterus, and placenta) were minced with a scalpel or scissors, swabbed with a sterile cotton swab, and streaked onto the culture media. All samples were spread onto Mueller-Hinton agar containing a *Campylobacter* selective supplement[e] (polymyxin B, rifampicin, trimethoprim, and cycloheximide) and a *Campylobacter* growth supplement[f] (sodium pyruvate, sodium metabisulphite, and ferrous sulphate) and were incubated for 48 hours in anaerobic jars under microaerobic conditions at 42°C. Following incubation, *Campylobacter*-like colonies on each plate were counted to determine the CFUs in each sample.

ELISA—An ELISA was used to determine the concentration of *C jejuni*–specific IgG antibodies in guinea pig sera. Microtiter plates[g] were coated with 100 µL of whole membrane components (approx 60 ng/well) of *C jejuni* IA3902 in coating buffer (sodium carbonate; pH, 9.6) and incubated overnight at 25°C. Plates were then incubated with a blocking buffer (PBS solution containing 2% milk, 2% bovine serum albumin, and 0.1% Tween-20) at 37°C for 1 hour. Serum samples were diluted in the blocking buffer to 1:100, then 100 µL of each dilution was added to individual wells. Duplicate wells were used for each sample. After incubation at 25°C for 2 hours, the plates were washed 3 times with the wash buffer (PBS solution containing 0.1% Tween-20). Polyclonal, horseradish peroxidase–labeled goat anti-guinea pig IgG[h] was diluted to 1:1,000 in the blocking buffer and added to the wells (100 µL/well). After incubation for 2 hours at 25°C, the plates were washed 3 times with the wash buffer before the horseradish peroxidase substrate[i] was added. Optical density values of individual wells were measured by use of an ELISA reader[j] at 405 nm.

Statistical analysis—A commercial statistical software package[k] was used to perform all analyses. A Fisher exact test for binomial variables was used when comparing positive and negative results of *Campylobacter* culture from samples of blood, liver, bile, uterus, and placenta. A 1-way ANOVA was used to detect differences in ELISA results obtained from sera, and a Tukey adjustment for multiple comparisons was made. Values of $P \leq 0.05$ were considered significant.

Results

Campylobacter spp were not isolated from any of the prechallenge rectal swab specimens. One sham-vaccinated guinea pig aborted approximately 36 hours after inoculation with *C jejuni* IA3902 and was euthanized and necropsied immediately. All remaining guinea pigs were euthanized 48 hours after inoculation, at which time clinical signs of impending abortion were not observed.

Gross lesions—At necropsy, gross lesions were limited to the liver and consisted of random, pinpoint to 2-mm-diameter white foci scattered throughout the parenchyma. These lesions were noted in 10 of 14 sham-vaccinated animals, 11 of 13 animals that received vaccine A, 2 of 12 animals that received vaccine B, and 2 of 12 animals that received the experimental bacterin.

Histopathologic lesions—Suppurative placentitis was detected in the junctional zone between the sub-

placenta and decidua in all but 1 placental sample with positive results of culture for *C jejuni* (7/8 samples) and in none of the placental samples with negative results of culture for *C jejuni* (0/11). Additionally, multifocal hemorrhage was present in the junctional zone of some affected placentas. Random multifocal suppurative hepatitis was a consistent finding in animals that received a sham vaccination or vaccine A. Lesions were most frequent and of the greatest severity in the group that received vaccine A. Rare, small suppurative infiltrates were observed in a few animals that received vaccine B or the experimental bacterin.

***Campylobacter* culture**—*Campylobacter jejuni* was recovered from at least 1 sample (blood, bile, liver, uterus, or placenta) in 26 of 51 animals. Of these 26 animals, 8 were pregnant and 18 were not pregnant. The frequency of positive results of bacteriologic cultures of blood and tissue was summarized by treatment group and by pregnancy status within treatment groups (**Table 1**). Compared with controls, animals that received vaccine B had significantly lower recovery of *Campylobacter* from blood ($P = 0.017$), bile ($P = 0.017$), liver ($P = 0.002$), uterus ($P = 0.043$), and placenta ($P = 0.048$). Animals that received the homologous bacterin had significantly lower recovery from the liver and bile ($P = 0.047$ and $P = 0.017$, respectively), compared with controls. Recovery rates for animals that received vaccine A did not differ significantly from controls. When comparing treatment groups by pregnancy status, the distribution of recovery rates was similar to that observed in each group overall; however, significance was often not maintained (possibly because of the small number of samples in certain subsets).

For all animals with demonstrable tissue infection, the quantity of *Campylobacter* organisms recovered from each sample type was summarized (**Table 2**). Overall, *Campylobacter* organisms were recovered in the highest numbers from the placenta of pregnant animals (8/8 with > 1,000 CFUs) and bile of nonpregnant animals (11/18 with > 1,000 CFUs). Although the recovery rates were generally low (< 50 CFUs), most infected animals (23/26) had positive results of bacteriologic culture of liver, independent of pregnancy status. For all samples except bile, organisms were recovered in greater quantity from pregnant animals.

ELISA results—Mean ELISA OD values for *Campylobacter*-specific antibody in sera from guinea pigs receiving vaccine A (OD = 1.53), vaccine B (OD = 1.07), and the experimental bacterin (OD = 1.68) were significantly greater ($P < 0.001$, $P = 0.003$, and $P < 0.001$, respectively), compared with the value for sham vaccination (OD = 0.44). Mean ELISA ODs for vaccine A and the experimental bacterin did not differ significantly ($P = 0.796$); however, the mean OD for vaccine B was significantly lower than that for the experimental bacterin and vaccine A ($P = 0.006$ and $P = 0.041$, respectively).

Vaccine efficacy—An animal was considered to have eliminated infection at 48 hours if all tested tissues had negative results of *Campylobacter* culture. Vaccine B yielded the highest overall proportion of animals free of infection, with 11 of 12 animals eliminating infection by 48 hours, and this differed significantly ($P < 0.001$) from sham-vaccinated controls in which only 3 of 14 had negative results of *Campylobacter* culture. A significantly greater percentage of animals free of infection was also observed with vaccine B when comparing the subgroups of pregnant ($P = 0.033$) or nonpregnant ($P = 0.026$) animals with controls. The overall clearance rate for animals that received the homologous bacterin was 9 of 12, which differed significantly ($P = 0.016$) from that of controls; however, this was not significant when pregnant ($P = 0.167$) or nonpregnant ($P = 0.106$) animals were compared separately with controls. For animals receiving vaccine A, there was no significant ($P > 0.99$ for all tests) difference in the elimination of infection overall (2/13 animals) or by pregnancy status, compared with sham-vaccinated controls.

Table 1—Proportions of bacteriologic cultures with positive results for blood and various tissues obtained 48 hours after IP inoculation with *Campylobacter jejuni* in guinea pigs of various experimental groups.

Groups and vaccines	Blood	Bile	Liver	Uterus	Placenta
All animals					
Sham vaccine	6/14	6/13*	10/14	5/14	3/3
Vaccine A	4/13	9/13	9/13	8/13	2/2
Vaccine B	0/12†	0/12†	1/12‡	0/12†	1/7†
Bacterin	1/12	0/12†	3/12†	2/12	2/7
Pregnant animals only					
Sham vaccine	2/3	0/2*	2/3	3/3	3/3
Vaccine A	2/2	2/2	2/2	2/2	2/2
Vaccine B	0/7	0/7	1/7	0/7‡	1/7†
Bacterin	1/7	0/7	2/7	2/7	2/7
Nonpregnant animals only					
Sham vaccine	4/11	6/11	8/11	2/11	—
Vaccine A	2/11	7/11	7/11	6/11	—
Vaccine B	0/5	0/5	0/5†	0/5	—
Bacterin	0/5	0/5	1/5	0/5	—

Values represent the number of samples with positive results of bacteriologic culture per number of samples tested.

*A bile sample was unavailable from 1 guinea pig in this group. †Value differs significantly ($P < 0.05$) from value for sham-vaccinated controls. ‡Value differs significantly ($P = 0.01$) from value for sham-vaccinated controls.

— = Not applicable. Vaccine A = A commercial polyvalent product labeled for prevention of campylobacteriosis in sheep via two 5-mL doses administered SC. Vaccine B = A commercial polyvalent product labeled for prevention of campylobacteriosis in sheep via two 2-mL doses administered SC.

Table 2—Semiquantitative values for recovery of *C jejuni* from necropsy samples obtained from guinea pigs not protected by vaccination against challenge with *C jejuni* IA3902.

	Sample source				
Groups	**Blood**	**Bile**	**Liver**	**Uterus**	**Placenta**
Pregnant animals					
Sham	+++	–	++	+++	+++
Sham	–	–	–	+	+++
Sham	+++	*	++	+++	+++
Vaccine A	+++	++	++	++	+++
Vaccine A	+++	+++	+++	+++	+++
Vaccine B	–	–	+	–	+++
Bacterin	+	–	+	++	+++
Bacterin	–	–	+++	++	+++
Nonpregnant animals					
Sham	–	–	+	–	
Sham	+	+++	+	–	
Sham	+	+++	+	–	
Sham	+	+	+	–	
Sham	+	+++	+	–	
Sham	–	+++	+	+	
Sham	–	+++	++	–	
Sham	–	–	+	+	
Vaccine A	–	++	+	–	
Vaccine A	+	+++	+	+	
Vaccine A	–	+++	++	–	
Vaccine A	+	–	–	–	
Vaccine A	–	+++	+	+	
Vaccine A	–	–	–	+	
Vaccine A	–	+++	+	+	
Vaccine A	–	+++	+	+	
Vaccine A	–	+++	+	+	
Bacterin	–	–	+	–	
Total positive (proportion)	11/26	15/25	23/26	15/26	8/8

*No sample available.
– = No growth. + = Low growth (< 50 CFUs). ++ = Moderate growth (50 to 1,000 CFUs). +++ = High growth (> 1,000 CFUs).
See Table 1 for remainder of key.

Discussion

Comparative vaccine efficacy studies against emergent pathogens and novel pathogen strains are vital for veterinary practitioners and producers to help guide product selection during the development of disease management strategies. Unfortunately, the cost and logistics of rapidly assessing vaccine efficacy in the target species are often prohibitive. Ideally, *Campylobacter* vaccine efficacy studies would be undertaken in sheep; however, the high seroprevalence for *Campylobacter* spp, high carriage rate of *Campylobacter* spp in the intestine and bile of healthy sheep,[26,27] high cost of *Campylobacter*-negative specific pathogen–free sheep, and lengthy gestation are substantial impediments to the assessment of multiple vaccine treatments in pregnant ewes. The goal of the present study was to design and evaluate a more manageable model for screening *Campylobacter* vaccines and to use this model in the assessment of the potential efficacy of 2 commercially available ovine-labeled *Campylobacter* vaccines[a,b] against an emergent *C jejuni* strain.

The small size, reduced housing costs, and ease of obtaining *Campylobacter*-negative animals make guinea pigs a desirable animal for evaluating the efficacy of *Campylobacter* vaccines. In previous studies,[19–22] prevention of abortion in pregnant guinea pigs following IP challenge has been the evaluation endpoint; however, if animals are to receive 2 doses of vaccine prior to inoculation, they need to be purchased at a time point prior to that at which pregnancy can be confirmed or be obtained from a large breeding colony. In either situation, it is unlikely that 100% of vaccinated animals will be pregnant or will successfully become pregnant and nonpregnant animals would be unusable if abortion is the sole evaluation endpoint. Additionally, the lengthy postinoculation observation period (up to 21 days) and any unused nonpregnant animals would further inflate the overall cost of the study. Pregnant guinea pigs are highly susceptible to *C jejuni* IA3902,[18] and high numbers of organisms are potentially recoverable from blood and other nonreproductive tissues (liver and bile) in addition to fetoplacental units as early as 48 hours after inoculation. Thus, the present study was designed to evaluate a mixed population of pregnant and nonpregnant guinea pigs in screening for *Campylobacter* vaccine efficacy, a process that has not previously been described.

In contrast to venereally acquired campylobacteriosis in cattle, sheep are more likely to acquire *Campylobacter* organisms via the oral route with subsequent bacteremia and seeding of fetoplacental units by abortifacient strains. As such, resolution of bacteremia following IP challenge was selected as a means of screening the efficacy of ovine campylobacteriosis vaccines. In the study reported here, elimination of infection was considerably higher in animals that received vaccine B (11/12) or the experimental homologous bacterin (9/12), compared with those that received vaccine A

(2/13) or a sham vaccination (3/14). This relative efficacy was maintained for each treatment independent of pregnancy status. The efficacy of vaccine B was significantly greater than that of the sham treatment when analyzed overall or between subsets of pregnant and nonpregnant animals, indicating a clear relationship between this product and resolution of infection with *C jejuni* IA3902. The homologous bacterin was slightly less efficacious than vaccine B, and a significant difference between the bacterin and controls was evident when comparing all animals treated; however, there was not a significant difference when pregnant subsets were compared, possibly because of the low number of animals in each subset. Administration of vaccine A failed to significantly reduce infection 48 hours after inoculation with IA3902 in any form of analysis.

Use of ELISA revealed that both commercial products and the experimental bacterin induced a significant concentration of *Campylobacter*-specific antibody relative to sham-vaccinated controls. This provided additional evidence of a difference in efficacy between the 3 treatments with regard to resolution of infection with *C jejuni* IA3902 because the specificity of the antibodies in each group appears to differ despite a similar quantitative antibody response. Vaccine B had a lower mean OD than either vaccine A or the experimental bacterin, yet it was associated with the highest level of efficacy on the basis of resolution of infection. Although it is tempting to infer this as evidence that the quality of antibody is more important than the quantity, it is possible that a greater percentage of antibodies produced in response to use of vaccine B were consumed during the resolution of infection, resulting in lower OD in the sera at 48 hours. Further study with preinoculation sera is necessary to fully evaluate the quantitative difference in antibody concentration among treatments; however, observing the greatest resolution of infection in the group with the lowest mean OD suggested that each treatment induced an adequate quantity of antibody and therefore vaccination failure was an unlikely source of the observed differences among groups.

Campylobacter jejuni was recovered from the blood, bile, liver, and uterus in pregnant and nonpregnant guinea pigs 48 hours after inoculation, revealing that nonpregnant guinea pigs had a similar overall tissue distribution relative to pregnant animals following IP inoculation with this organism. In nonpregnant animals, the greatest relative quantity of *C jejuni* was recovered from the bile, whereas in pregnant animals, the placenta was the tissue associated with highest rate of recovery with considerably less recovery from the bile. Previous studies reveal that *C jejuni* is attracted to the mucin component of bile[28] and that certain bile acids induce virulence gene expression in *C jejuni*.[29] Thus, identifying *C jejuni* in the bile was not surprising. However, the differential predilection for the bile in nonpregnant guinea pigs versus the placenta in pregnant animals observed in the present study was intriguing given that a recent study[30] revealed that *C jejuni* is driven by energy taxis and seeks conditions most favorable for growth. Results of the present study may therefore suggest the presence of a placental factor more chemoattractive than bile that drives the placental tropism observed with this organism. Further study involving the tropic effects of individual placental factors is necessary to further elucidate the nature of placental tropism with *C jejuni*.

Results of the present study indicated that evaluation of blood and tissue infection in pregnant and nonpregnant guinea pigs 48 hours after IP inoculation can effectively be used to screen potential *Campylobacter* vaccines in a shorter, more cost-effective manner than that previously described. By use of this model, administration of 2 doses of vaccine B to pregnant and nonpregnant guinea pigs significantly reduced infection following IP inoculation with *C jejuni* IA3902, comparable with that of a homologous bacterin, but administration of vaccine A was ineffective despite inducing a significant concentration of *Campylobacter*-specific antibody. This underscores the importance of efficacy testing against emergent pathogenic strains and provides further evidence of inadequate cross protection between heterologous *C jejuni* strains. These findings also suggest the presence of common protective antigens shared by the strains included in vaccine B and the challenge strain IA3902. Further studies such as immunoblotting with representative sera from the different groups may provide clues to the reasons for this observation. Because *C jejuni* IA3902 has recently been described as the predominant ovine abortion-associated isolate in multiple states,[4] protection against this emergent strain should be an essential component of effective campylobacteriosis disease management programs, and the added fact that IA3902 is highly resistant to tetracycline increases the importance of vaccination in disease prevention. Given the remarkably high proportion of guinea pigs receiving vaccine B that were free of infection 48 hours after inoculation with a quantity of *C jejuni* IA3902 reported to induce abortion in 100% of IP inoculated animals (11/12),[18] it is likely that vaccine B would be the most effective presently available commercial vaccine for controlling ovine campylobacteriosis caused by this specific strain; however, these findings do not necessarily reflect the effectiveness of either commercial product against the broad spectrum of *Campylobacter* spp that sheep may encounter in the field. Additionally, the high level of efficacy observed with the experimental homologous bacterin in this study suggests that in vaccinated flocks experiencing abortion caused by an unspecified strain of *Campylobacter*, an autogenous product may be an appropriate consideration.

a. *Campylobacter fetus* bacterin (serial No. 1369B), Colorado Serum Co, Denver, Colo.
b. *Campylobacter fetus–jejuni* bacterin (serial No. 06-251), Hygieia Laboratories, Woodland, Calif.
c. Elm Hill Labs, Chelmsford, Mass.
d. Aluminum hydroxide gel (13 mg/mL), Sigma-Aldrich, St Louis, Mo.
e. Preston *Campylobacter* selective supplement, Oxoid Ltd, Cambridge, England.
f. *Campylobacter* growth supplement, Oxoid Ltd, Cambridge, England.
g. Nunc-Immune plate, Nunc, Roskilde, Denmark.
h. Anti-Guinea Pig IgG (H+L), peroxidase labeled, KPL, Gaithersburg, Md.
i. ABTS 2-Component Microwell Peroxidase Substrate, KPL, Gaithersburg, Md.

j. FLUOstar Omega Microplate Reader, BMG Labtech Inc, Durham, NC.
k. SAS, version 9.2, SAS Institute Inc, Cary, NC.

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